Improved Determination of Aluminum in Serum and Urine with Use of a Stabilized Temperature Platform Furnace

Fred Y. Leung and Arthur R. Henderson

This method for determining aluminum in serum and urine is essentially free from matrix interference and gives a linear response with concentration to at least 500 µg/L. Use of a stabilized temperature platform (L'vov platform, Perkin-Elmer Corp.) to approach a "steady-state" temperature, addition of matrix modifiers [especially Mg(NO₃)₂], and the use of peak area integration all helped substantially diminish spectral interference. With the platform furnace, serum protein concentrations as great as 260 g/L did not interfere with the determination of Al. The within- and between-assay precision (CV) was ≤3.5% and ≤7.4%, respectively. Analytical recovery of Al added to serum ranged between 95 and 101% throughout the linear calibration range (to 500 µg/L), either when measured directly from the standard curve or by the method of standard additions. The reference interval for Al in 28 healthy subjects was 2–14 µg/L (mean 6.5, SD 4.1 µg/L), and for 130 patients on hemodialysis, 20–550 µg/L (mean 87.5, SD 62.5 µg/L).

Additional Keyphrases: trace elements • electrothermal atomic absorption • reference interval • hemodialysis patients

Aluminum is ubiquitous, being the most common metal in the earth's crust. Although Al toxicity in humans appears to be rare, in patients on maintenance hemodialysis for end-stage renal failure Al accumulates in brain, bone, and liver. It also is implicated as a possible causative agent for an encephalopathy (dialysis dementia), for a type of osteomalacia, and for microcystic hypochromic anemia (1–3).

The use of various analytical methods—spectrophotometry, emission spectrometry, neutron activation, and atomic absorption spectrometry—has produced widely divergent values, ranging from 2 to 1900 µg/L, for Al in serum of apparently healthy subjects (4). Incorporating technological improvements of the past decade, a sensitive and simple technique for measurement of Al in serum and urine is reported here that gives results linear with concentration to at least 500 µg/L.

We use graphite-furnace atomic absorption spectroscopy with the stabilized temperature furnace (L'vov platform) and sample-matrix modification. The results so obtained are compared with those by use of the usual graphite tube without the platform.

Materials and Methods

Instrumentation

Unless otherwise specified, all the special instrumentation is from Perkin-Elmer Corp., Norwalk, CT 06852. To determine Al in serum and urine, we used a microprocessor-controlled double-beam atomic absorption spectrophotometer (Model 5000), a graphite furnace complete with programmer (Model HGA 500), and an auto sampler complete with sequencer (Model AS40). Atomization signals are traced with a chart recorder (Model 56). The light source was an Al hollow-cathode lamp, used in conjunction with a deuterium arc lamp to correct for background noise. We used pyrolytically coated graphite tubes (no. 091504) with solid pyrolytic graphite platforms (no. 02902311). The platform and graphite tube assembly are diagrammed in Figure 1A. This commercially available platform, with a sample capacity of 50 µL, is similar to that described by Slavin and Manning (5).

An insertion tool is shown in Figure 1B, and the correct positioning directly below the sample port in the centre of the graphite tube is shown in Figure 1C. In routine analysis for Al in serum or urine, we used a 10-µL sample, dispensed with the auto sampler. Sample probe and platform should be carefully adjusted so that the sample is injected only onto the platform. The platform, heated by radiation from the furnace wall, produces an atmosphere during sample atomization that approaches equilibrium temperature conditions (6).

![Fig. 1. A, Design of the L'vov platform in scale dimensions. B, Positioning of the platform with insertion tool. C, Final ready position for sample, and perspective view with cut-open furnace (not in scale).](image-url)
Standards
A 1000 μg/L (1000 ppm) atomic absorption Al reference solution in dilute aqua regia is used (Canlab, Toronto, Ontario, M5Z 2H4 Canada). Aliquots of this stock are diluted with purified water to yield working standards containing between 10 and 500 μg of Al per liter. Purified water with a specific resistance >18 MΩ/cm is obtained with the Milli-RO and Milli-Q purification systems (Millipore Ltd., Mississauga, Ontario, L4V 1L2 Canada).

Unless otherwise specified, all containers are soaked in a saturated solution (about 0.5 mol/L) of disodium ethylene-diaminetetraacetate (Na2 EDTA) for as long as 4 h, then rinsed several times with purified water.

Samples
Blood is collected in red-top Vacutainer Tubes (Becton Dickinson Co., Rutherford, NJ 07070), which are essentially free from contamination with Al. After clotting and centrifugation at 1200 × g for 10 min at room temperature, the resulting serum layer is transferred to Na2 EDTA pre-rinsed polypropylene tubes (no. 2059; Falcon, Oxnard, CA 93030). For storage and transport of samples for Al analyses, screw-cap 7-mL polypropylene tubes are suitable; they are free of detectable Al as supplied (W. Sarstedt, St. Laurent, Quebec, H4S 1P5 Canada).

Sera obtained randomly from patients admitted to this hospital and having a low Al content (<2.5 μg/L) were pooled and used as matrix in samples measured to prepare calibration curves. The pooled serum was aliquoted into 1-mL portions and stored frozen (−20 °C) until use. Before assay, the serum samples were diluted with an equal volume of a solution containing, per litre, 1.4 g of magnesium nitrate [Mg(NO3)2], “Suprapur” grade; E. Merck, Darmstadt, F.R.G.) and 2 mL of Triton X-100 (“Scintillation” grade; BDH Chemicals, Toronto, Ontario, M5Z 1K5 Canada).

Urine samples are collected in Na2 EDTA pre-rinsed polypropylene containers without preservatives. Before analysis, they are stored at 4 °C for no longer than 24 h, or at −20 °C for longer periods. The urine samples are diluted with an equal volume of purified water for analysis. A linear calibration line is obtained when Al standards up to 500 μg/L, in a pooled specimen of urine (<2.0 μg of Al per liter) as the matrix, are analyzed.

In the determination of urine Al, a deuterium arc lamp background corrector is necessary to reduce baseline noise during the atomization step.

Procedure
For determination of serum or urine Al concentrations, the following conditions are used:

Spectrophotometer settings. Aluminum hollow-cathode lamp current 25 mA; wavelength, 309.3 nm; low window slit, 0.7 nm; integration time, 6 s; AA-BO mode (sample absorbance minus background).

Graphite furnace program. The basic settings used for the graphite furnace without and with the L’vov platform are given in Table 1. Argon flow is 300 mL/min during all steps except during atomization, when it is 10 mL/min. When the platform is used, a clean-up cycle at 2700 °C for 4 s and a ramped cool-down cycle of 20 s are also added.

Results and Discussion
Furnace conditions. The choice of optimum conditions for the electrothermal atomization of Al depends on the ability of the system to attain isothermal conditions with minimal spectral interferences. With the standard graphite tube, the sample is deposited onto the wall of the tube, which must be heated slowly to avoid splattering. When samples are vaporized from the hot wall surface into a cooler gaseous atmosphere, vapor-phase matrix interference can occur (7). This spectral interference is more pronounced at the cooler end regions of the graphite tube where a vapor “fog” forms (6).

With the use of the standard graphite tube, our optimum conditions (Table 1) are very similar to those reported by Gardiner et al. (8). We also use a two-step ramped charring procedure, at 700 and 1500 °C, to minimize interference from components of the matrix. A background peak noted at 600 °C was removed with the ramp step to 700 °C. Inorganic matrix interference between 800 and 900 °C (8) appeared to be removed from our system by the added matrix modifiers—1 mL of Triton X-100 surfactant and 0.7 g of Mg(NO3)2 per liter—and by the use of ramp heating to 1500 °C. Above 1600 °C, some Al is lost. A temperature of 2700 °C is used for the atomization step, because the signal-to-noise ratio is better than at higher temperatures.

Many of the spectral and matrix interferences are decreased or minimized by use of the stabilized temperature platform furnace (9). Because this platform is heated from the tube wall by radiation, the rate of temperature increase in it is more gradual and linear. As a result, ramp heating is not necessary, but a longer hold time is required to achieve the stabilized temperature conditions. Using maximum-power atomization, a lower temperature (2500 °C) is used with the platform than with the standard furnace alone (Table 1).

Interferences. An advantage of using graphite furnace atomization is that no sample pre-treatment is needed before injection into the furnace. Al in serum, however, is a matrix-analyte combination that results in a nonlinear calibration curve above 100 μg/L, as illustrated by the dashed line in Figure 2. Oster (10), using a standard graphite tube, obtained a linear standard curve to 100 μg of Al per liter. Measurement of higher Al concentrations would necessitate several-fold dilution of the samples to bring them within the linear range of the method, and would require correspondingly diluted calibration standards to match the matrix changes.

Sometimes a reagent can be added to the sample, to produce a chemical change during drying or charring or both. The function of these matrix modifiers may be either to decrease
the volatility of the analyte, so as to prevent volatilization during the charring step, or to increase its removal at this stage (11). Use of Mg(NO₃)₂ at a final concentration of 2.5 g/L as a matrix modifier in the determination of Al in aqueous solutions has been described earlier (12). We tested the effect of this modifier in concentrations ranging from 0.7 to 2.5 g/L for determination of Al in serum. These samples were analyzed with the usual graphite tube. As observed with the added matrix modifier, there was some improvement in linearity, but concentrations of Al > 200 μg/L were essentially still nonlinear (Figure 2).

The addition of the stabilizing temperature platform to the graphite furnace improved the linearity of the calibration range. A difference in peak shape and appearance time between sampling off the platform rather than off the wall of the furnace requires the use of peak area integration (7). The use of peak area integration with the platform increased linearity only up to 200 μg/L. The addition of chemical modifiers, however, significantly extended linearity up to at least 600 μg/L with an absorbance of 1.0. Because absorbance error can increase at this concentration (13), we chose to measure the concentration to 500 μg/L, which gave an absorbance range up to 0.8 unit (Figure 3). The double-beam monochromator in the spectrophotometer reduces stray light to 0.01%, and this source of error would not be a significant factor in causing absorbance deviations. The addition of Triton X-100 to the samples, as described by Kaehny et al. (14), decreased residue buildup on the platform. A 0.7 g/L concentration of Mg(NO₃)₂ was chosen, to allow the best detection sensitivity for low concentrations of Al. In a recent electrothermal atomic absorption method in which an aerosol-generating device is used to apply Al samples, King et al. (15) reported a linear calibration response to at least 400 μg/L.

**Table 2. Effects of Serum Proteins on Results for Aluminum**

<table>
<thead>
<tr>
<th>Concentration of Serum Protein, g/L</th>
<th>Absorbance at 308.3 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concn of Al, μg/L</td>
</tr>
<tr>
<td></td>
<td>65</td>
</tr>
<tr>
<td>25</td>
<td>0.08</td>
</tr>
<tr>
<td>50</td>
<td>0.13</td>
</tr>
<tr>
<td>100</td>
<td>0.22</td>
</tr>
<tr>
<td>200</td>
<td>0.39</td>
</tr>
<tr>
<td>300</td>
<td>0.57</td>
</tr>
</tbody>
</table>

Serum pools with added Al standards. Values are an average of two experiments, with CVs <4%.

*Effects of proteins.* In serum, Al is bound to nondialyzable plasma constituents such as proteins (16). To assess the effect of various serum protein concentrations on our assay, we lyophilized normal serum proteins, which then were reconstituted and added to standard aqueous solutions of Al. Table 2 shows the absorbance measurements at three concentrations of the serum proteins—65, 130, and 260 g/L—with concentrations of Al between 25 and 300 μg/L. Each point represents an average of two experiments, with each determined in triplicate. We saw no significant difference in absorbance.
between samples with a normal concentration of proteins and those containing above-normal concentrations. Evidently, protein is effectively removed in the charring step.

Method assessment. To assess the accuracy of the method, we supplemented pooled serum samples (Al concentrations <2.5 μg/L) with standards containing Al concentrations of 10 to 500 μg of Al per liter. The samples, with optimized concentrations of the matrix modifiers added [0.7 g of Mg(NO₃)₂ and 1 mL of Triton X-100 per liter], were analyzed with use of the L'vov platform. With use of the direct microprocessor-calculated concentration method, with the basal serum concentration subtracted by the auto-zero function, the net analytical recovery of the Al added to the serum pool ranged between 95 and 101% throughout the range to 500 μg/L. Good agreement with this recovery was also obtained by reading the absorbance and calculating the results by the method of standard additions.

We assessed the precision of our procedure by preparing our own reference sera at three concentration ranges of Al. Portions of these samples were stored at −20 °C for use in monitoring our daily analyses. Table 3 shows the results for these precision studies, both within- and between-assays. The within-run precision shown is typical of that for an analysis done in a graphite furnace with a useful half-life of about 150 firings. The condition of the rods can vary to produce exceedingly good precision (CV <1.0%) or less satisfactory variations (CV >10.0%). When the higher variations are observed the analysis is repeated, perhaps after changing the graphite tube. In contrast, the L'vov platform has a much longer useful life—at least five times the number of firings obtainable (>1500) with our currently used graphite tubes. Although the between-run precision is slightly higher (CV 3.4–7.4%) it is satisfactory for clinical use.

The sensitivity of the method with use of the platform is such that 2.2 μg of Al per liter produced an absorbance of 0.0044 with a serum matrix. Similar responses are obtainable with urine samples, although background correction with the deuterium arc lamp is necessary to achieve these results.

The reference interval for aluminum in serum from healthy persons (n = 28) was determined to be 2–14 μg/L (mean 6.5, SD 4.1 μg/L), results that agree with values recently reported (8, 10, 17). By comparison, a random sampling of 130 patients on renal dialysis gave serum Al concentrations in the range of 20–550 μg/L (mean 87.5, SD 62.5 μg/L), values corresponding to the range for hemodialysis patients of 8–713 μg/L (mean 87.7 μg/L) reported by Oster (10) and the range of 50–600 μg/L for renal-dialysis patients found by Gardiner et al. (8).

We compared this method with another graphite furnace atomic absorption method (8) by analyzing 10 specimens in two laboratories. The Al concentrations in these specimens ranged from 16 to 270 μg/L; the correlation coefficient (r) was 0.97. The equation for the correlation is y = 0.73x + 0.83, where x is the present L'vov platform method and y is the method of Gardiner et al. (8).

Urinary Al can be determined by a similar method as used for serum Al. With the L'vov platform, linearity up to 500 μg/L was obtained. The analysis of urine did not require the chemical matrix modification we used for serum, but the deuterium arc lamp background corrector was necessary for baseline stability. Urinary aluminum in normal subjects was less than 10 μg/L as determined by our procedure. These values are similar to the normal 24-h excretion of 13 ± 5 μg reported by Kaehny et al. (16).

The improved performance for the determination of serum and urine Al concentrations requires careful integration of several factors. These include optimized furnace variables for the system used, the appropriate type and amount of matrix modifiers, and the ability of the instrument rapidly to record and quantitate the absorption signal. The procedure described here is used routinely in our hospital for the analysis of patients' samples.

References

Liquid-Chromatographic Determination of Amitriptyline and Its Metabolites in Serum, with Adsorption onto Glass Minimized

**Peter M. Edelbroek, Ed J.M. de Haas, and Frederik A. de Wolff**

To study correlations between the concentrations, in serum, of amitriptyline and its most important metabolites with clinical response in patients, we developed a "high-performance" liquid-chromatographic method for routine determination of amitriptyline, nortriptyline, total 10-hydroxy-amitriptyline, desmethylamitriptyline, and E(trans)- and Z(cis)-10-hydroxyamitriptyline. These compounds are extracted from 1 mL of alkalized serum into hexane/isoamyl alcohol (99/1 by vol). Perazine is the internal standard. To minimize irreversible adsorption of the drugs onto the glassware, 5 μg of maprotiline is added to the organic phase just before evaporation. After a 10-min resolution on a silica column eluted with acetonitrile/methanol/NH₄OH (1 mol/L), absorbance is measured at 240 nm. Only chlorpromazine, doxepin, procaainamide, and N-acetylprocaainamide may interfere with assays of the compounds that probably are therapeutically relevant: amitriptyline, nortriptyline, and E-10-hydroxyamitriptyline. Uremia, lipemia, and icterus also do not affect the analysis.

Additional Keyphrases: drug assay • analytical error • sample handling • monitoring therapy • tricyclic antidepressants • dosage-compliance test

The tricyclic compound amitriptyline (AT) ¹ is widely used to treat mental depression. It is extensively metabolized (φ), mainly by N-demethylation, hydroxylation, and N-oxidation (Figure 1).

Results from animal studies (2-3) indicate that not only are AT and NT `active, but also their 10-hydroxy metabolites may have antidepressive activity in man.

Methods for assay of AT and other tricyclic antidepressants were recently reviewed (4-5). In only one gas-chromatographic–mass spectrometric method (6) and a few HPLC methods (7-9) could AT and NT and its hydroxylated metabolites be determined. Moreover, the procedures reported (6-9) are laborious and incomplete; e.g., drug interferences were not tested. For several tricyclics, irreversible adsorption onto glass has been described. Efforts (4, 5, 10-13) to prevent adsorption—such as silanization and siliconizing of glassware, replacement by plastic, pretreatment of glassware with alkylamines or alkyl alcohols, or addition of these reagents to the solution for evaporation—gave variable results. To assess correlation between concentrations of AT and its most important metabolites in serum with clinical response in patients, we developed an HPLC method for determination of the drug and its metabolites, including a simple and effective procedure to minimize irreversible adsorption of the drugs onto the glassware.

Materials and Methods

**Drugs and reagents.** All chemicals were of analytical grade. Hexane, isoamyl alcohol, methanol, sodium hydroxide, and ammonium hydroxide were purchased from E. Merck AG, Darmstadt, F.R.G.; acetonitrile (Chromat grade) from Mallinkrodt, Inc., Paris, KY 40361. AT HCl, NT HCl, AT-NO, E-10-OH-AT, Z-10-OH-AT, E-10-OH-NT, Z-10-OH-NT, and DNT HCl were kindly supplied by Lundbeck & Co., Amsterdam, The Netherlands. Perazine dimaleate was a gift from Bijk, Zwanenburg, and maprotiline from Ciba-Geigy, Arnhem, The Netherlands.

We prepared stock solutions of AT, its metabolites, and perazine dimaleate in ethanol to give a concentration of 1 g/L in terms of the free base. Maprotiline was used as a 2.5 g/L solution in methanol. Serum standards of 25, 50, 100, 200, and 300 μg/L were prepared by adding AT, NT, Z-10-OH-AT, DNT, E-10-OH-NT, and Z-10-OH-NT to drug-free pooled serum, then stored at -20 °C in 1-mL aliquots in conical glass tubes with Teflon-lined screw-caps.

**Glassware.** The tubes and their caps were washed with laboratory detergent (Liquinox; Alconox Inc., New York, NY 10003), cleaned with dichromate-sulfuric acid, rinsed with tap water and distilled water, and dried.

For collecting blood samples and concentrating the extract, we used disposable conical glass tubes without further cleaning.

**Apparatus.** The chromatograph used was a laboratory-assembled instrument consisting of: a pump of reciprocating piston-type with flow feedback control (Model 740; Spectra Physics, Mountain View, CA 94042), an ultraviolet detector with wavelength variable (GM 770; Schoeffel, Westwood, NJ 07675), and a syringe-loading sample injector with a 175-μL loop and variable injection volume (Model 7105; Rhodyne Cotati, CA 94928). For all separations we used a 10 × 0.3 cm column, packed in our laboratory with 5-μm (av. particle di-